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Blood Schizonticidal Activity of *Phyllanthus amarus* Enhances Testovarian Antioxidant Defense Capacity in *Plasmodium berghei* Infected Mice

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ABSTRACT

In the present study, the crude ethanol leaf extract of *Phyllanthus amarus* was investigated for its *in vivo* activity against *Plasmodium berghei* malarial parasite in both early and established infections. The study also evaluated the changes in testicular and ovarian antioxidant defense capacity in *P. berghei* infected mice treated with the *P. amarus* leaf extract (100, 200 and 300 mg/kg) using biomarkers (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) and malondialdehyde (MDA)) and documental procedures. Results show increased ($p < 0.05$) ovarian levels of antioxidants (GSH, SOD, CAT and GPx) with insignificant change in MDA of *P. berghei* infected mice when compared with control suggesting good antioxidant capacity. Testes of the *P. berghei* infected mice, however, showed a significant decrease ($p<0.05$) in the levels of the antioxidant with an associated increase in MDA ($p<0.05$) amounts when compared with control values. These data indicate that the testes are more susceptible to oxidative stress than the ovaries during malarial infection. Blood schizonticidal activity of *P. amarus* exhibits repository actions against *P. berghei* parasites in a dose-dependent manner as Groups 1, 2, 3 and 4 show established infection values of 0.0, 61.0 ± 8.0, 72.0 ± 6.0 and 87.0 ± 10, respectively. Also, *P. amarus* administration whether in the presence or absence of *P. berghei* infection significantly boosts antioxidant defense capacity and hence invigorated the testes and ovaries as indicated by the empirical data and histopathological evidence. The active phytochemicals, however, need to be identified for further study.

Keywords: Testes, ovaries, schizonticidal, *Phyllanthus amarus*, *Plasmodium berghei*.

Introduction

Malaria is an infectious disease caused by a single-celled parasite belonging to the genus *Plasmodium*. There are more than 100 different species but *Plasmodium falciparum* is the deadliest among humans.[1] Malaria threatens the lives of more than one-third of the world's population and it remains a major killer of humans worldwide. The disease exists mainly in the tropical areas of Asia, Africa, and America, where it infects millions of people. Each year, 350 to 500 million cases of malaria occur worldwide[2] and more than 1 million of its victims, mostly young children and pregnant women, die annually.[2,3]

It is known that malarial infection is accompanied by increased production of reactive oxygen species (ROS) and the malarial parasites are sensitive to oxidative damage.[3] The univalent reduction of oxygen results in a series of cytotoxic oxygen species such as superoxide anions (O_2^{-}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^{-}).[4] These highly ROS can cause a wide-spectrum of cell damage including lipid peroxidation, inactivation of enzymes, alteration of intracellular oxidation-reduction state and damage to DNA.[5-7]

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Mammalian cells, however, possess antioxidant activities which protect the body from the damaging effects of oxygen free radicals.[4] Although several antioxidant enzymes are essential in this defense system, the most important include glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD). These enzymes act directly on some free radicals, making them less reactive. However, they are not able to act on the highly reactive free radicals such as hydroxyl and peroxynitrite which are chiefly responsible for oxidative and pathological processes. As such, the body uses small molecules that reduce the reactivity of various reactive radicals as an auxiliary antioxidant defense system. This group contains numerous number of molecules, such as vitamins (A, C and E), beta-carotene, uric acid and reduced glutathione (GSH) molecule.[8]

A potential source of free radical production in malaria is the host's haemoglobin molecule. The parasite uses this molecule as a source of amino acids for its own nutrition during the erythrocytic stage of the disease, resulting in the liberation of large amounts of circulating heme. By having Fe^{2+}-associated groups, these heme groups are able to induce intravascular oxidative stress, causing changes in erythrocytes and endothelial cells and facilitating the internalization of the parasite in tissues such as the liver and brain.[8,9] Though much has been achieved towards the eradication of malaria, it still remains a major source of concern due to compelling circumstances hampering the full control of environmental factors, the mosquito vectors and emerging drug resistance.[10] Therefore, it is necessary to search for novel and effective anti-malarial agents including those derived from herbs.

Herbs have always formed an integral part of human health and so, are used in the treatment of several human diseases. One of such herbal plant is *Phyllanthus amarus*. *P. amarus* is widely used and it has been shown to possess anticancer,[11] antiamnesic,[12], antinociceptive,[13] antimicrobial[14,15], antileptospiral[16] activities in addition to its antiplasmodial[17] and antioxidant potentials.[17-19] These observed activities may be due to the

phytochemical constituents (alkaloids, flavonoids, tannins, saponins, anthroquinones, carbohydrate) already identified in the plant.[20,21]
Thus, the present study attempts to establish a correlation between the effects of antioxidant activities of the plant on its antimalarial activity in the testes and ovaries of *Plasmodium berghei* infected mice treated with *P. amarus* ethanolic leaf extract.

Materials and Methods

Harvesting and preparation of leaf extract
Fresh plants of *Phyllanthus amarus* were collected from natural habitat in Ozoro, Isoko North LGA of Delta State, Nigeria between February to March 2015. The plant was identified at the Nigeria Institute of Forestry Research, Ibadan, where a voucher specimen (No: FHI109728) has been deposited. The leaves were washed, air-dried and ground into fine powder. The powder (500 g) was soaked in 70% ethanol overnight and then extracted using a Soxhlet apparatus. The extract was evaporated to dryness using a rotary evaporator (Buchi R-210, Hana China) under reduced pressure. The percentage yield was 3.6%. The dried extract obtained was dissolved in distilled water to a concentration of 2.5 mg/mL. The volume administered in millilitre (mL) equivalent to dose (mg/mL) studied was then calculated thus:

V (mL) = D (g/kg) x P (kg)/C (g/mL)

D= dose used (g/kg b.wt); P= body weight (kg); C= concentration of the extract (g/mL); V=volume of extract (mL) administered.

Experimental animals
Forty-five (45) adult Swiss albino mice of mixed sexes weighing between 20 to 30 g were obtained from the Laboratory Animal Centre, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria and used for the study. Three (3) *Plasmodium berghei* infected (donor) mice were obtained from the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria.

Animal care and handling
The mice were fed on growers' mash obtained from Top-Feeds, Sapele, Delta State, Nigeria, and were given clean drinking water *ad libitum*. The animals were kept in plastic cages, under controlled condition of 12 h light/12 h dark cycle. The animals were maintained in accordance with the guidelines approved by the Animal Ethical Committee, Faculty of Basic Medical Sciences, Delta State University, Abraka.

Animal grouping, inoculation and extract administration
The forty-five (45) animals were grouped into nine (n = 5/group) to allow easy movement and to avoid crowdedness. Group 1 was inoculated with phosphate buffered saline (PBS) (pH 7.4) and treated with placebo-normal saline (normal control); Group 2 was infected with *Plasmodium berghei* (PB) and treated with placebo-normal saline (malaria control); Groups 3, 4 and 5 were parasitized (infected with PB) and treated with 100, 200 and 300 mg/kg b.wt of *P. amarus* ethanolic leaf extract, respectively; Group 6 was also parasitized (infected with PB) but treated with 5 mg/kg b.wt of chloroquine. Group 7, 8 and 9 were inoculated with PBS and treated with 100, 200 and 300 mg/kg b.wt of *P. amarus* ethanolic leaf extract, respectively. The *P. amarus* ethanolic leaf extract and the chloroquine doses were administered as single daily dose using intragastric cannula for a period of seven (7) days.
The mice were infected by obtaining parasitized blood (3-4 drops) from the cut tail tip of an infected (donor) mouse. Then, 0.1mL of infected blood was diluted in 0.9mL of phosphate buffer, pH 7.2 and the mice were inoculated with 0.1 mL of the parasitized suspension which contained about ten thousand (10,000) parasites. Parasitaemia was confirmed after 72 hrs using thin blood films prepared from the cut tail tip of the infected mouse and stained with Giemsa stain (1%), then, viewed with the microscope (Model TH-9845, Serico, China) at x100 magnification as reported by Cheesbrough.[22]

Collection of specimen and analysis from experimental animals
On the 7th day, the experimental animals were fasted overnight and sacrificed under chloroform anaesthetic. Ovaries and testes were collected for biochemical analysis and histopathological examination.

Histological examination
Tissues (testes and ovaries) were fixed with Bouin's solution. They were later sectioned using a microtome and dehydrated in graded alcohol. The dehydrated tissues were embedded in paraffin and thereafter stained with Hemotoxylin and Eosin (H and E). The stained tissue slides were mounted under a microscope connected to a computer for views.

Biochemical analysis
The testes and ovaries collected were subsequently weighed and twenty percent tissue homogenate prepared using normal saline under cold conditions. The crude tissue homogenate was centrifuged at 2000 rpm for 15 min at 4°C). The supernatant collected was stored at -20°C until required for biochemical analysis.

Antioxidant capacity of specimen
The ovaries and testes of mice in the various test groups were analyzed to assess the antioxidant capacity of *P. amarus* during malarial infection. Formation of malondialdehyde (MDA) was estimated by determining the accumulation of thiobarbituric acid reactive substances (TBARS)[23] in the homogenates while the level of reduced glutathione was assessed using the method of Moron *et al.*[24] The activities of glutathione peroxides,[25] superoxide dismutase,[26] and catalase[27] were determined using the colorimetric method.

Statistical analysis
Experimental data in five replicates were expressed as Mean ± SD (Standard Deviation). Results were subjected to statistical analysis using one-way analysis of variance (ANOVA) to determine the differences between groups. Differences between means at 5% level ($p < 0.05$) were considered significant.

Results and Discussion

The results obtained from this investigation into the schizonticidal activity of *Phyllanthus amarus* and associated changes in testicular and ovarian antioxidant defense capacity in *Plasmodium berghei* infected mice treated with *P. amarus* ethanolic leaf extract show that *P. berghei* malarial parasite infection induced oxidative stress and hence damage in the ovaries (Table 1) and testes (Table 2) of experimental animals. Introduction of *P. amarus* boosted the antioxidant defense capacity of these tissues in a dose-dependent manner. In addition, treatment with *P. amarus* and standard chloroquine dose show schizonticidal activity in both early and established infectious conditions (Table 3). Onyesom *et al.*[17] and Dapper *et al.*[28] demonstrated the antiplasmodial activity of *P. amarus*. Several other medicinal plants such as *Pyrenacantha staudtii*,[29] *Aspilia africana*,[30] *Amaranthus spinosus* L. and *Andrographis paniculata* Burm. f./Nees,[31] *Piper betle*[32] and *Acacia auriculiformis*[33] have also been shown to possess antimalarial activity. Chloroquine treatment did not augment the amounts of antioxidants as *P. amarus* treatment and so could not protect the testes and ovaries against free radical challenge as evidenced by the MDA values (Tables 1 and 2) and Photomicrographs (Figures 1-18). Therefore, the identified compounds in the phytochemical profile of *P. amarus*[17] contain phytoconstituents with both antiplasmodial and antioxidant activities.
Several researches have validated the antioxidant potential of *P. amarus*. Kumar and Kuttan[34] showed that *P. amarus* methanolic extract increased antioxidant defense mechanism in mice and protected the animals from radiation-induced cellular damage. Arun and Balasubramanian,[35] Syed *et al.*[36] and Padmaja *et al.*[37] showed the hepatoprotective value of *P. amarus*. Reduction in testicular activities of the antioxidant enzymes as well as levels of GSH with a significant increase in the level of MDA as observed in this study is in line with the work of Nnodim and Nwanjo.[38] The decrease in antioxidants could be associated with free radicals induced by the *Plasmodium* parasites. Reactive oxygen species can interact with antioxidants and inactivate antioxidant enzymes producing a state of oxidative stress. The decrease in SOD and CAT activities could be attributed to oxidative inactivation of enzymes.[39] The high MDA observed in the present study could bond with amino group of proteins to form intra- and inter-molecular crosslinks which could inactivate several membrane-bound enzymes. The increased levels of MDA in testes and ovaries of *P. berghei* infected mice (Group 2, Tables 1 and 2) may indicate damage triggered by free radicals.[40] Lipid peroxidation (LPO) is a degenerative mechanism of membrane component mediated through free radical production in the cell.[41]

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Table 1: Changes in ovarian antioxidants and malondialdehyde levels in *Plasmodium berghei* infected mice treated with *Phyllanthus amarus* and chloroquine

Group	SOD (units/mg protein)	CAT (µmole H_2O_2/min/mg protein)	GPx	GSH (mg/g tissue)	MDA (units/g tissue)
1	0.82 ± 0.37	12.72 ± 1.52	7.22 ± 0.82	12.32 ± 2.10	35.10 ± 3.70
2	1.38 ± 0.07	20.23 ± 2.84*	11.05 ± 1.34*	22.72 ± 2.04*	38.94 ± 3.51
3	1.30 ± 0.20	17.04 ± 1.70*	8.82 ± 0.19*	17.70 ± 1.20*	14.75 ± 2.13*
4	1.55 ± 0.08*	21.96 ± 2.94*	10.64 ± 1.51*	17.70 ± 3.63*	9.88 ± 0.52*
5	2.74 ± 0.72*	17.28 ± 1.66*	10.30 ± 1.36*	18.64 ± 1.50*	7.14 ± 1.34*
6	0.75 ± 0.47	12.26 ± 1.58	3.78 ± 0.83*	6.30 ± 2.30*	38.98 ± 6.33
7	1.42 ± 0.12	18.78 ± 1.22*	9.34 ± 0.46	16.32 ± 4.79	8.00 ± 1.60*
8	1.39 ± 0.10	20.30 ± 2.69*	9.46 ± 0.80*	18.04 ± 1.58*	9.40 ± 1.56*
9	1.98 ± 0.30*	21.70 ± 3.21*	14.20 ± 2.12*	19.32 ± 2.34*	4.18 ± 1.44*

SOD, Superoxide dismutase; CAT, Catalase; GPx, Glutathione peroxidase; GSH, Reduced glutathione, MDA, Malondialdehyde. *Significant at ($p < 0.05$) when compared with normal control values.

Table 2: Changes in levels of antioxidants and malondialdehyde in testes of mice infected with *Plasmodium berghei* and treated with *Phyllantus amarus* and chloroquine.

Group	SOD (units/mg protein)	CAT (µmole H_2O_2/min/mg protein)	GPx	GSH (mg/g tissue)	MDA (units/g tissue)
1	1.03 ± 0.16	12.66 ± 1.54	6.10 ± 0.84	12.62 ± 1.64	44.10 ± 1.31
2	0.07 ± 0.03	10.92 ± 0.96	2.26 ± 0.74	1.38 ± 0.24*	75.45 ± 0.07*
3	1.61 ± 0.41	13.10 ± 2.17	11.68 ± 0.59*	19.50 ± 3.51*	7.62 ± 1.73*
4	1.77 ± 0.50	18.38 ± 3.16	15.32 ± 2.59*	20.54 ± 2.71*	6.90 ± 1.97*
5	2.76 ± 0.73	27.93 ± 6.71*	25.14 ± 2.96*	19.40 ± 1.72*	4.76 ± 1.66*
6	6.30 ± 1.74*	42.13 ± 3.82*	41.97 ± 2.80*	34.40 ± 3.78*	3.32 ± 1.34*
7	5.66 ± 2.03*	38.14 ± 3.15*	53.40 ± 3.73*	42.74 ± 2.82*	4.24 ± 1.38*
8	5.16 ± 1.41*	35.34 ± 3.53*	63.84 ± 3.41*	37.30 ± 2.61*	2.38 ± 0.85*
9	8.20 ± 1.49*	44.10 ± 3.51*	64.17 ± 3.35*	55.42 ± 3.03*	1.62 ± 0.62*

SOD, Superoxide dismutase; CAT, Catalase; GPx, Glutathione peroxidase; GSH, Reduced glutathione, MDA, Malondialdehyde. *Significant at ($p < 0.05$) when compared with normal control values.

Table 3: Blood schizonticidal activity of *Phyllanthus amarus* in early and established *P. berghei* infection in mice.

Group	Blood schizonticidal activity of *Phyllanthus amarus* (%)	
	Early infection	Established infection
1	-	-
2	0.0	0.0
3	52.0 ± 4.0	61.0 ± 8.0
4	58.0 ± 6.0	72.0 ± 6.0
5	64.0 ± 5.0	87.0 ± 10
6	68.0 ± 7.0	89.0 ± 8.0
7	-	-
8	-	-
9	-	-

SOD, Superoxide dismutase; CAT, Catalase; GPx, Glutathione peroxidase; GSH, Reduced glutathione, MDA, Malondialdehyde. *Significant at ($p<0.05$) when compared with normal control values.

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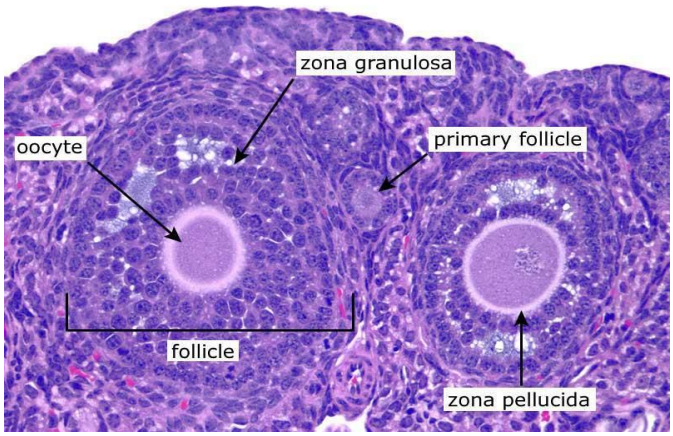

Figure 1: Histology of ovary of the control mice (Group 1). Features and ovarian cell appear normal. Microscopic magnification = x100, (H and E staining).

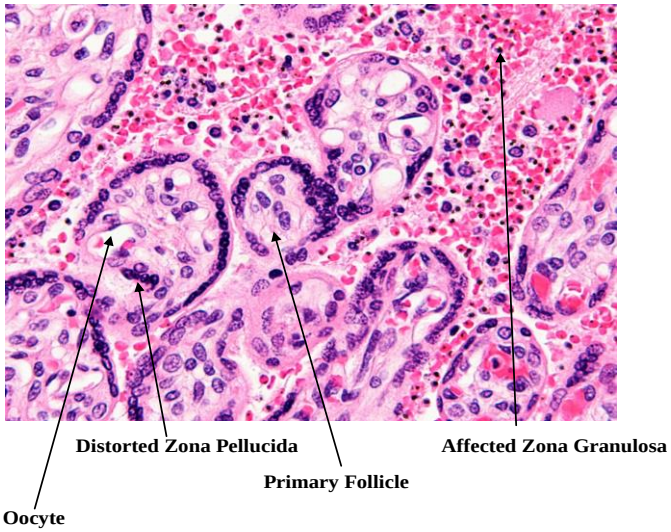

Figure 2: Histology of ovary cells infected with *P. berghei* (Group 2). Pathological feature show distorted linings of the zona pellucida, the primary follicle and the zona granulosa. Microscopic magnification = x100, (H and E staining).

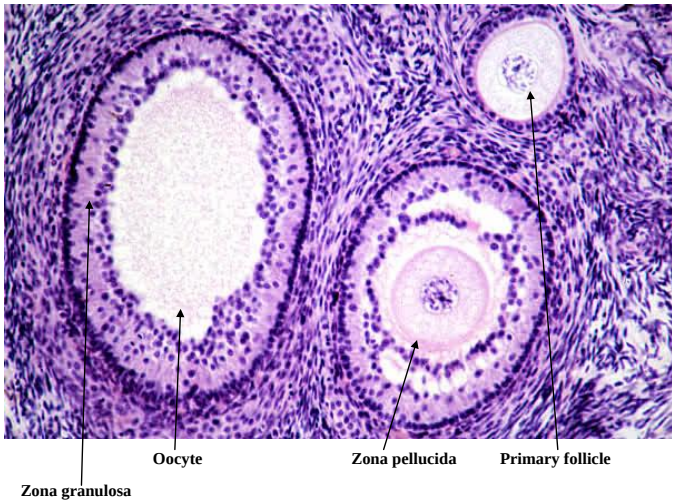

Figure 3: Histology of ovarian cells infected with *P. berghei* and treated with 100 mg/kg *Phylllantus amarus* for 7 days (Group 3). Features show a mild improvement in the distorted lining of the zona pellucida and zona granulosa. Microscopic magnification = x100, (H and E staining).

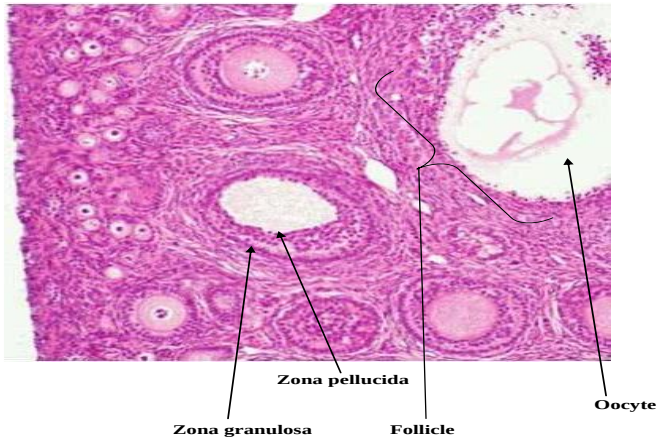

Figure 4: Histology of ovarian cells infected with *P. berghei* and treated with 200 mg/kg *Phylllantus amarus* for 7 days (Group 4). Features indicate moderate improvement in the lining of the zona pellucida and zona granulosa, an evidence of repair and recovery. Microscopic magnification = x100, (H and E staining).

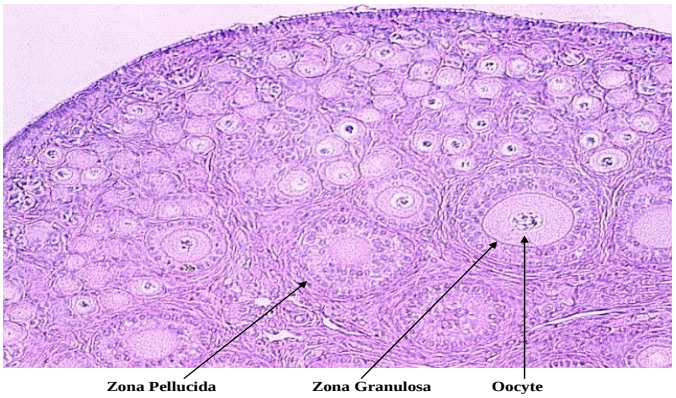

Figure 5: Histology of ovarian cells infected with *P. berghei* and treated with 300 mg/kg *Phylllantus amarus* for 7 days (Group 5). The zona pellucida and zona granulosa appear perfectly normal. This dose of *P. amarus* protects ovarian cells from oxidative assault by *P. berghei*. Microscopic magnification = x100, (H and E staining).

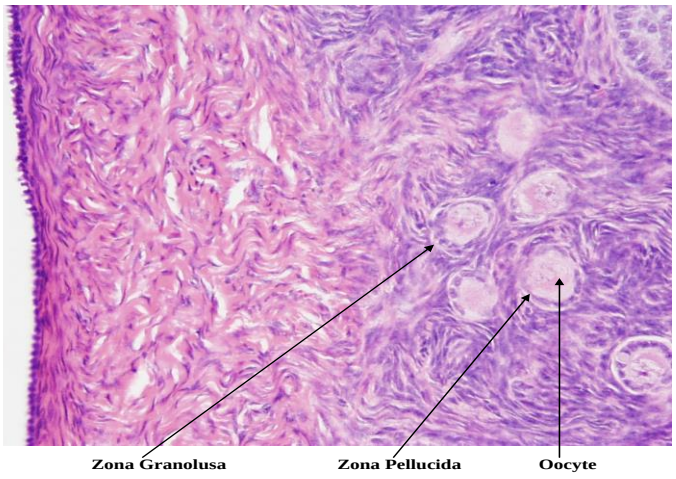

Figure 6: Histology of ovarian cells infected with *P. berghei* and treated with 5 mg/kg chloroquine for 7 days (Group 6). Features show moderate distortion in the linings of the zona pellucida and zona granulosa. Microscopic magnification = x100, (H and E staining).

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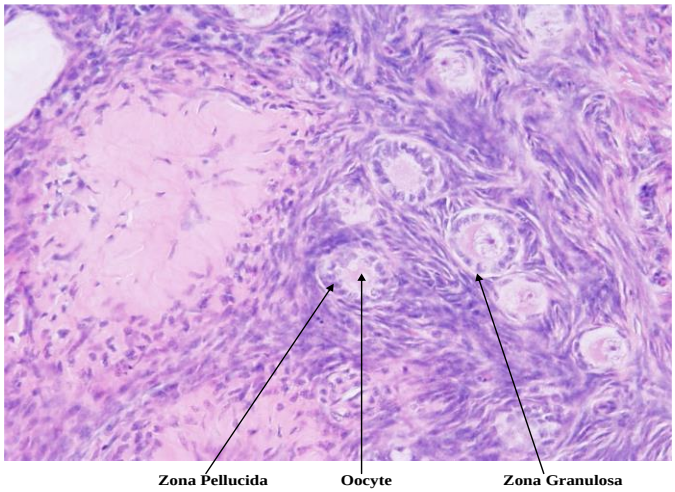

Figure 7: Histology of ovarian cells not infected but treated with 100 mg/kg of *Phyllantus amarus* (Group 7). Features appear normal when compared with the control. Microscopic magnification = x100, (H and E staining).

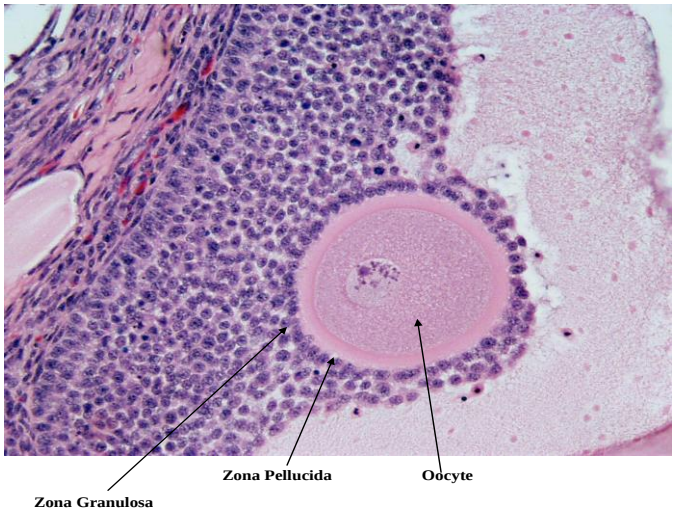

Figure 8: Histology of ovarian cells not infected but treated with 200 mg/kg of *Phyllantus amarus* (Group 8). Features show cells replenishment. Microscopic magnification = x100, (H and E staining).

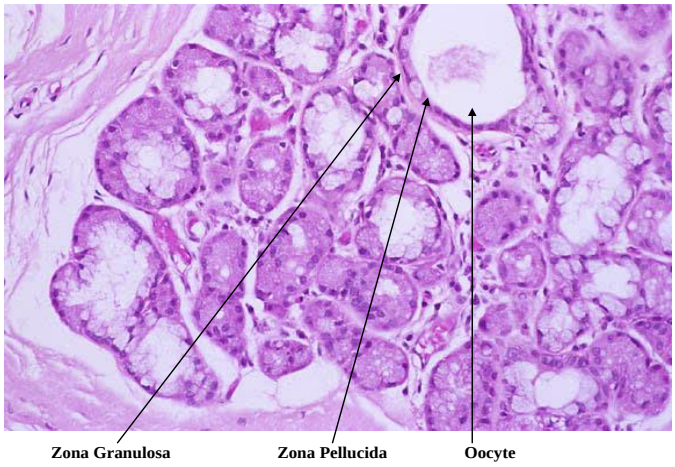

Figure 9: Histology of ovarian cells not infected but treated with 500 mg/kg of *Phyllantus amarus* (Group 9). Cell features appear normal and invigorated when compared with the control. Microscopic magnification = x100, (H and E staining).

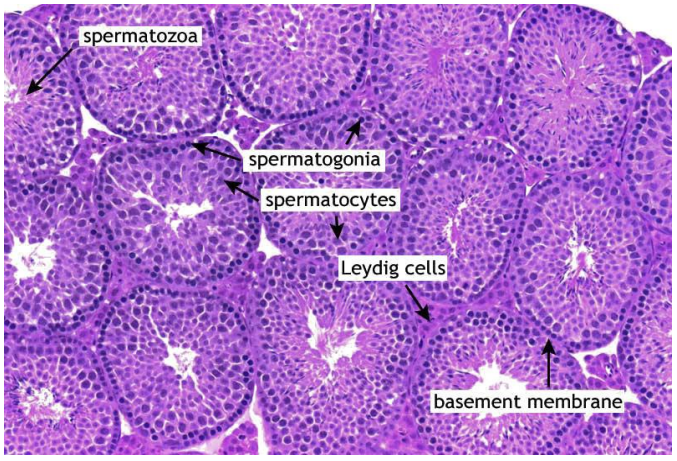

Figure 10: Histology of testes for control mice (Group 1). Features indicate normal linings of the spermatogonia, spermatocytes, leydig cells and the basement membrane. Microscopic magnification = x100, (H and E staining).

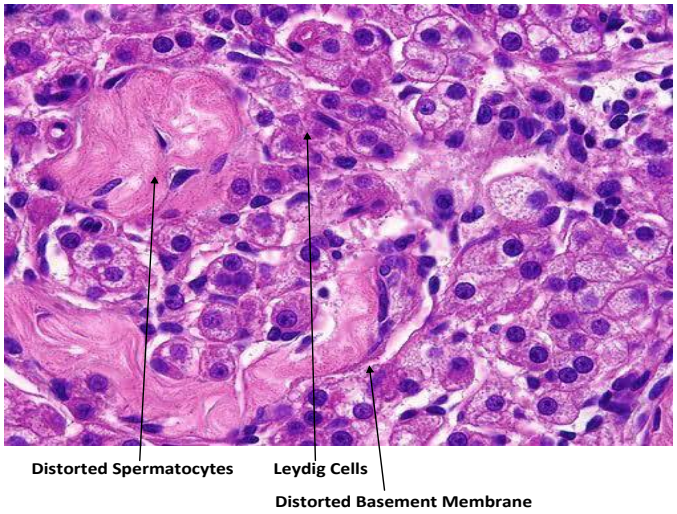

Figure 11: Histology of testes for mice infected with *P. berghei* (Group 2). Features show total distortion in the lining of the basement membrane, the spermatogonia, spermatocytes and leydig cells. Microscopic magnification = x100, (H and E staining).

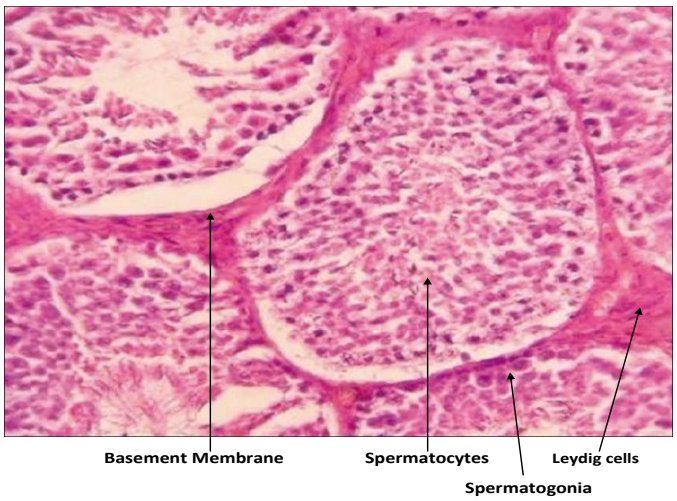

Figure 12: Histology of testes for mice infected with *P. berghei* (Group 3) and treated with 100 mg/kg of *Phyllantus amarus* for 7 days. Features indicate mild changes in the tissue. Microscopic magnification = x100, (H and E staining).

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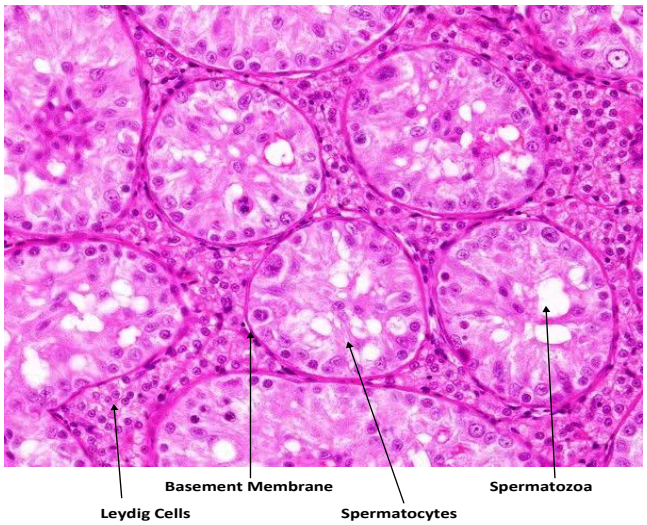

Figure 13: Histology of testes for mice infected with *P. berghei* (Group 4) and treated with 200 mg/kg of *Phyllantus amarus* for 7 days. Features indicate significant improvement in the tissue distortions when compared with *P. berghei* infection only. Microscopic magnification = x100, (H and E staining).

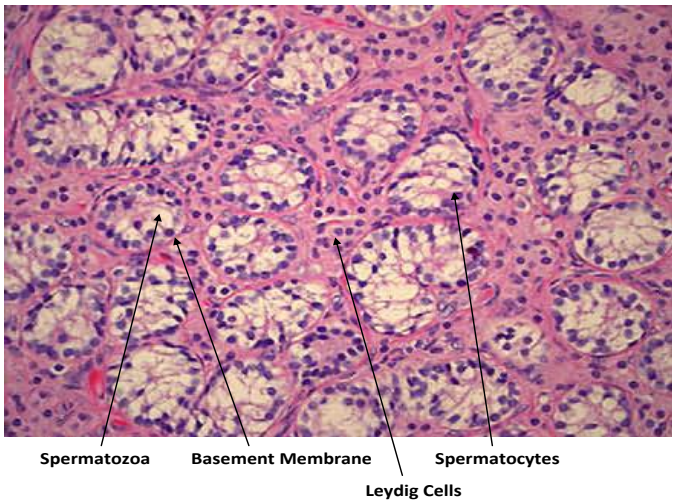

Figure 14: Histology of testes for mice infected with *P. berghei* (Group 5) and treated with 300 mg/kg of *Phyllantus amarus* for 7 days. Features of the cell appeared normal when compared with the control group. Microscopic magnification = x100, (H and E staining).

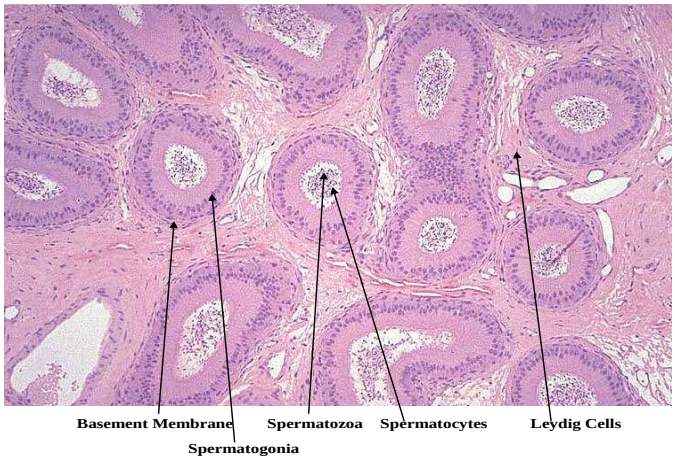

Figure 15: Histology of testes for mice infected with *P. berghei* (Group 6) and treated with chloroquine for 7 days. Features show moderate distortion in the cells of the testes when compared with the control. Microscopic magnification = x100, (H and E staining).

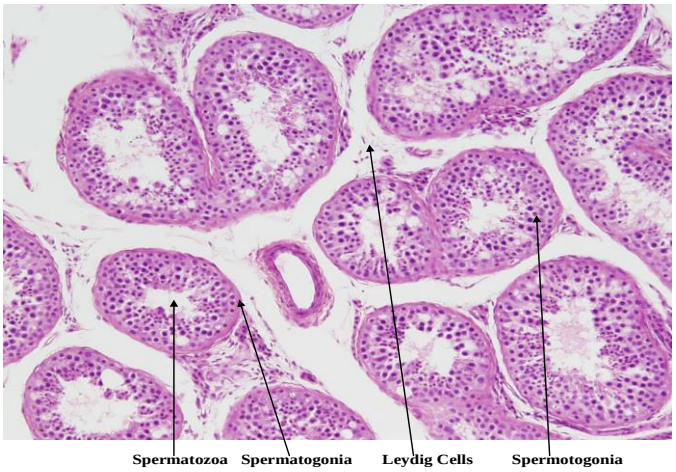

Figure 16: Histology of testes for mice without *P. berghei* infection (Group 7) but administered with 100 mg/kg *Phyllantus amarus* for 7 days. No damage was observed in the tissue. Microscopic magnification = x100, (H and E staining).

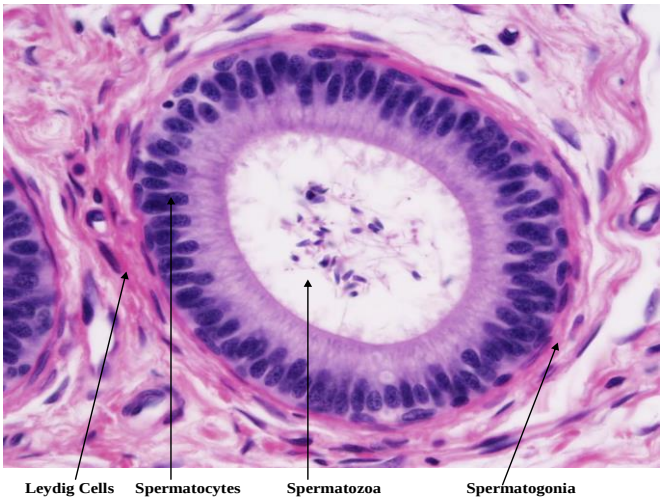

Figure 17: Histology of testes for mice without *P. berghei* infection (Group 7) but administered with 200 mg/kg *Phyllantus amarus* for 7 days. Cell features appear normal. Microscopic magnification = x100, (H and E staining).

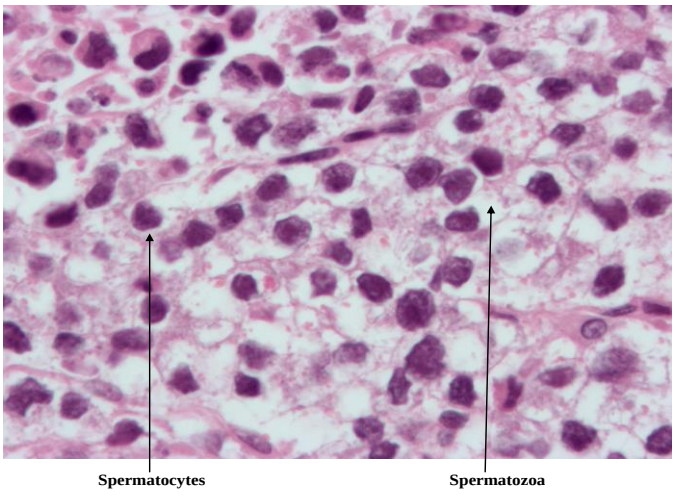

Figure 18: Histology of testes for mice without *P. berghei* infection (Group 7) but administered with 300 mg/kg *Phyllantus amarus* for 7 days. Cell were protected and replenished. Microscopic magnification = x100, (H and E staining).

Increased LPO implies membrane instability which correlates with altered tissue membrane as observed in the present study. In the ovarian cells, infection with *P. berghei*, results in the generation of highly reactive oxygen species (ROS). However, *P. amarus* was shown to be able to greatly nullify this effect. It was able to increase the activity of the antioxidant enzymes necessary for the reduction of the already produced ROS. This is in line with the study of Kumar and Kuttan[33] who reported increases in the activities of SOD, CAT, GST, GPx, glutathione reductase(GR) in both blood and tissue which were initially reduced by radiation treatment.

P. amarus has been shown to possess several active ingredients.[17] A variety of tannins purified from *P. amarus* was found to be potent inhibitors of protein kinases.[42] The presence of several lignans such as phyllantin and hypophyllantin, polyphenols, flavonoids and some ellagitannins isolated from *P. amarus*[17,43] could also be responsible for the antioxidant activity exhibited by the extract. Many plant phenolic compounds show excellent antioxidant activities and are good inhibitors of lipid peroxidation. Although the exact mechanism of *P. amarus* action needs to be elucidated, the combined action of these components could be responsible for its antioxidant and antiplasmodial properties.

Conclusion

The current study has shown the antioxidant and antiplasmodial potential of *P. amarus*. *P. amarus* exerts its antioxidant effect by counteracting the formation or reactivities of free radicals induced by *P. berghei* infection via participation of the plant's phytochemicals. This offered a measure of protection to the ovaries and testes of plasmodium parasites infected mice. However, the mechanism of *P. amarus* antimalarial potential involving its chemical compounds is still emerging.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Butzloff S, Groves MR, Wrenger C, Muller BI. Cytometric quantification of singlet oxygen in the human malaria parasite *Plasmodium falciparum*. Cytometry Part A 2012; 81A:698-703.
2. Dwivedi S, Dwivedi A, Kaul S. Evaluation of antimalarial herbs drugs. Pharm Lic Network 2007. 1-2 p.
3. Farombi EO, Shyntum YY, Emerole GO. Influence of chloroquine treatment *and Plasmodium falciparum* malaria infection on some enzymatic and non-enzymatic antioxidant defense indices in humans. Drug Chem Toxicol. 2003; 26(1):59-71.
4. Tyagi A, Tyagi R, Vekariya R, Ahuja A. Study of antioxidant enzymes, MDA and lipid profile in cerebral malaria. Ind J Clin. Pract. 2013; 23(12):823-825.
5. Das BS, Patnaik JK, Mohanty S, Mishra SK, Mohanty D, Satpathy SK, Bose TK. Plasma antioxidants and lipid peroxidation products in falciparum malaria. Am J Trop Med Hyg. 1993; 49:720–725.
6. Akanbi OM, Omonkhua AA, Cyril-Olutayo CM, Fasimoye RY. The antiplasmodial activity of *Anogeissus leiocarpus* and its effect on oxidative stress and lipid profile in mice infected with *Plasmodium berghei*. Parasitol Res. 2012; 110:219–226.
7. Balla G, Jacob HS, Balla J, Rosenberg M, Nath K, Apple F, Eaton JW, Vercelloti GM. Ferritin: A cytoprotective antioxidant stratagem of endothelium. J Biol Chem. 1992; 267:18148–18153.
8. Percario S, Moreira DR, Gomes BAQ, Ferreira MES, Gonçalves ACM, Laurindo PSOC, Vihena TO, Dolabela MF, Green MD. Oxidative stress in malaria. Int J Mol Sci. 2012; 13:16346–72.
9. Kumar S, Bandyopadhyay U. Free heme toxicity and its detoxification systems in human. Toxicol Lett. 2005; 157:175–188.
10. Raghavendra K, Barik TK., Reddy BP., Sharma P, Dash AP. Malaria vector control: from past to future. Parasitol Res. 2011; 108:757-79.
11. Rajeshkumar NV, Joy KL, Kuttan G, Ramsewak RS, Nair MG, Kuttan R. Antitumour and anticarcinogenic activity of *Phyllanthus amarus* extract. J Ethnopharmacol. 2002; 81(1):17-22.
12. Joshi H, Parle M. Pharmacological evidence for antiamnesic potentials of *Phyllanthus amarus* in mice. Afri J Biomedical Res. 2007; 10:165.
13. Itoro E, Ukana D, Ekaete D. Phytochemical screening and nutrient analysis of *Phyllanthus amarus*. Asian J Plant Sci and Res. 2013; 3:116-122.
14. Oluwafemi F, Debiri F. Antimicrobial effect of *Phyllanthus amarus* and *Parquetina nigrescens* on *Salmonella typhi*. Afr J Biomed Res. 2008; 11(2):215-219.
15. Saranraj P, Sivasakthivelan P. Screening of antibacterial activity of the medicinal plant *Phyllanthus amarus* against urinary tract infection causing bacterial pathogens. Appl J Hyg. 2012; 1(3):19-24.
16. Chandan S, Umesha S, Balamurugan V. Anti-leptospiral antioxidant and DNA damaging properties of *Eclipta alba* and *Phyllanthus amarus*. Sci Rep. 2012; 1(4):1-8.
17. Onyesom I, Onumaechi IF, Ehiwario J, Dagana R. Antiplasmodial activity of *Phyllanthus amarus* preserves renal function in *Plasmodium berghei* infected mice. EJMP 2015; 5(1):109-116.
18. Verma S, Sharma H, Garg M. *Phyllanthus amarus*: A Review. J Pharmacogn Phytochem. 2014; 3 (2):18-22.
19. Sen A, Batra A. The study of *in vitro* and *in vivo* antioxidant activity and total phenolic content of *Phyllanthus amarus* Schum Thonn: A medicinally important plant. Int J Pharm Pharm Sci. 2013; 5:947.
20. Nwaoguikpe RN, Ujowundu CO, Braide W, Obi J. *Phyllantus amarus* (Schum. and Thonn): An Antiplasmodial Plant. Br J Med Med Res. 2014; 4(35):5494-5508.
21. Patel JR, Tripathi P, Sharm V. *Phyllantus amarus* ethnomedicinal uses, phytochemistry and pharmacology, a review. J Ethnopharmacol. 2011; 138(2):286-313.
22. Cheesbrough M. District Laboratory Practice in Tropical Countries. Part 1. Cambridge University Press, London. 1998. 239-258 p.
23. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Ana Biochem. 1979; 95:351-358.
24. Moron MA, DePierre JW, Mannevick B. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat liver. Biochem Biophys Acta 1979; 582:67-68.
25. Jakoby WB. Enzymatic Basis of Detoxication. 1st Edn., Academic Press, New York, 1980. 415 p.
26. McCord, JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem. 1969; 244:6049-6055.
27. Goldblith SA, Proctor BE. Photometric determination of catalyst activity. J Biol Chem. 1950; 187:705-709.
28. Dapper DV, Aziagba BN, Ebong OO. Antiplasmodial effects of the aqueous extract of *Phyllantus amarus* Schumach and Thonn against *Plasmodium berghei* in swiss albino mice. Niger J Physiol Sci. 2007; 22(1-2):19-25.
29. Olorunniyi, OF, Morenikeji OA. *In vivo* antimalarial activity of crude aqueous leaf extract of *Pyrenacantha staudtii* against *Plasmodium berghei* (NK65) in infected mice. Afr J Pharm Pharmacol. 2014; 8(12):342-345.
30. Okokon JE, Nwidu LL, Essiet GA. Evaluation of *in vivo* antiplasmodial activity of *Aspilia africana*. Inter J Pharmacol. 2006; 2(3):348-351.
31. Susantiningsih T, Rahmawati R, Prijanti AR, Sadikin M, Freisleben H. Schizonticidal effect of a combination of *Amaranthus spinosus* L. and *Andrographis paniculata* Burm. F./Nees extracts in *Plasmodium berghei*-infected mice. Med J Indones. 2012; 21:66-70.

32. Abdulelah H, Al-Adhroey ZMN, Hesham MA, Adel AA, Rohela M. Antimalarial activity of methanolic leaf extract of *Piper betle* L. Molecules 2011; 16:107-118.
33. Okokon JE, Jackson O, Opara KN, Etim E. *In vivo* antimalarial activity of ethanolic leaf extract of *Acacia auriculiformis*. Int J Drug Dev Res. 2010; 2(3):482-487.
34. Kumar HKB, Kuttan R. Protective effect of an extract of *Phyllanthus amarus* against radiation-induced damage in mice. J Rad Res. 2014; 45:133-139.
35. Arun K, Balasubramanian U. Comapartive study on hepatoprotective activity of *Phyllanthus amarus* and *Eclipta prostrata* against alcohol induced in albino rats. Int J Environ Sci. 2011; 2(1):373-391.
36. Syed AB, Iqbal MM, Kiranmai M, Ibrahim M. Hepatoprotective activity of *Phyllanthus amarus* seeds extracts in CCl4 Treated Rats: *In vitro* and *in vivo*. Global J Med Res. 2012; 12(6):38-49.
37. Padmaja SN, Shashikant VN, Ajit VS, Chitra CK. Role of *Phyllanthus amarus* treatment in hepatitis-C. Biomed Res. 2011; 22(3):319-322.
38. Nnodim JK, Nwanjo HU. Glycohaemoglobin concentration and hepatocellular enzymes activities in malaria patients in Owerri, Nigeria. Al Ameen J Med Sci. 2012; 5(2):25-27.
39. Nnodim JK, Nwanjo HU, Okolie NJ, Opara AU, Nwosu DC, OKoroiwu I, Dike J, Okorie H, Nwadike CN, Uduji HI. Erythrocytic Antioxidant Enzymes, Antioxidant Vitamins And Plasma Malondiadehyde In Malaria Infected Patients In Owerri. Aus J Basic Appl Sci. 2012; 6(8):365-368.
40. Pabón A, Carmona J, Burgos LC, Blair S. Oxidative stress in patients with non-complicated malaria. Clin Biochem. 2003; 36:71–78.
41. Veena CK, Josephine A, Preetha SP, Varalakshmi P. Effect of sulphated polysaccharides on erythrocyte changes due to oxidative and nitrosative stress in experimental hyperoxaluria. Hum Exp Toxicol. 2007; 26:923–932.
42. Polya GM, Wang BH, Foo LY. Inhibition of signal regulated protein kinases by plant derived hydrolysable tannins. Phytochem. 1995; 38:307-314.
43. Foo LY. Amarinic acid and related ellagitannins from *Phyllanthus amarus.* Phytochem. 1995; 39:217 - 224.